For this analysis, we implemented the commonly used bivariate probit model, with the correlation in liability defined as $\rho_g = \alpha_g A + C$, where α_g is 1/4 for half-siblings and 1/2 for full siblings. *A* and *C* are additive genetic and shared environment effects, subject to the restriction that $A + C < 1$. Although in IAN, data are not available on the precise allocation of time spent by maternal half-sibs in the household of the proband, we allowed for sharing of rearing environment as mothers overwhelmingly maintain legal guardianship and primary custodianship of maternal half siblings through the time of the usual age of onset of ASD.

We replicated these findings in an independent sample of ASD-affected families oversampled for minority status in the US Autism Centers of Excellence data collection (Supplementary Table 1). The sample included 132 full siblings, 41 maternal half siblings and 31 paternal half siblings known to have been reared in the household of their respective ASD-affected proband. In this study, all siblings were screened for current ASD symptomatology using the Social Responsiveness Scale[7] and affectation status was confirmed using the Autism Diagnostic Interview-Revised.[8] Recurrence rates in this replication sample, 0.073 for maternal half siblings and 0.114 for full siblings, were remarkably consistent with those derived from the larger IAN sample. This analysis thus addresses several limitations of the IAN data set including: under-representation of minority subjects, the possibility of incomplete ascertainment of case status when relying on community diagnosis and the unavailability of data regarding time spent in the household of the proband.

To our knowledge this is the first report of the half-sibling: full-sibling recurrence ratio from a large population of families affected by autism. We note that in the two largest published clinical twin studies of autism,[1,3] involving, respectively, 277 twin pairs ascertained in the same manner and from the same registry described here (IAN) and 192 twin pairs from the California Twin Registry, recurrence rates on the order of 0.30 were reported for male-male dizygotic twin pairs—over double what we observed here for non-twin siblings. The discrepancy between the recurrence rate for dizygotic twins and the rate for non-twin siblings (as reported here and elsewhere[5,9]) remains unexplained and requires further study to confirm and conclusively document the magnitude of the difference, but suggests the possibility that one mechanism by which genetic variation might incur risk for ASD in a fetus is through elicitation of deleterious maternal antibodies or other circulating factors, which—in twins but not sib pairs—could theoretically incur risk to the co-twin (irrespective of the genetic susceptibility status of the latter).

Given that an estimated 10–25% of the remaining causal variance is explainable on the basis of *de novo* mutation,[10] these data substantially contribute to the information base on the genetic structure of autism and strongly support a major role of genetic factors in the ontogeny of ASD, including the intergenerational transmission of additive genetic susceptibility factors through unaffected mothers.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Deep resequencing and association analysis of schizophrenia candidate genes

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In 2005, we selected 10 genes for which there was reasonable evidence for involvement in the etiology of schizophrenia (*COMT*, *DAOA*, *DISC1*, *DRD2*, *DRD3*, *DTNBP1*, *HTR2A*, *NRG1*, *SLC6A3* and *SLC6A4*, Supplementary Table S1).[1] Although these genes have not received support from far larger and comprehensive subsequent studies, and may not contain common etiological variations,[2] it is possible that they contain uncommon variations of etiological importance. To test this hypothesis, we conducted a multistage resequencing study.

In stage 1, we used Sanger methods to sequence the exons, 5′ and 3′ untranslated regions, splice sites, promoters and conserved intronic regions of these 10 genes in 727 cases with schizophrenia from CATIE[3] and 733 controls of European (EUR) and African (AFR) ancestry. In stage 2, we validated single-nucleotide variants (SNVs) using Roche 454 sequencing in the same samples. In stage 3, we genotyped prioritized SNVs in independent samples (Supplementary Material and Supplementary Figure S1).

In stage 1, Sanger sequencing identified 782 variants, including 587 novel SNVs that are not found in dbSNP132 (Supplementary Tables S2–S4). As expected, the number of novel variants discovered per individual was higher in those of AFR (1.46) than EUR ancestry (0.95), but cases and controls did not differ (EUR cases/controls: 0.920/0.980; AFR cases/controls: 1.492/1.430). The numbers of SNVs per gene were also similar, although *DISC1*

Table 1. Summary of stage 3 replication genotyping results

Gene	Single-marker analysis (57 SNVs)			Gene-based analysis (35 SNVs)				
	No. of SNVs	EUR (minimum P value)	AFR (minimum P value)	No. of uncommon variants	EUR (no. of case/control alternative alleles)	EUR P values (CALPHA/VT)	AFR (no. of case/control alternative alleles)	AFR P values (CALPHA/VT)
COMT	4	0.037	0.128	3	8/1	0.02/0.003	0/3	0.29/0.71
DAOA	5	0.039	0.121	0	—	—	—	—
DISC1	22	0.099	0.081	20	32/40	0.67/0.33	39/32	0.27/0.08
DRD3	3	0.375	0.264	0	—	—	—	—
DTNBP1	5	0.390	0.106	5	18/19	0.32/0.72	7/4	0.34/0.16
HTR2A	3	0.037	0.380	1	3/13	0.03/0.7	0/1	0.71/0.71
NRG1	8	0.119	0.017	1	4/1	0.14/0.1	159/185	0.34/0.15
SLC6A3	4	0.339	0.326	2	4/4	0.6/0.8	0/1	0.76/0.67
SLC6A4	3	0.418	0.310	3	10/9	0.45/0.25	1/1	0.4/0.36

Abbreviations: AFR, African; EUR, European; SNVs, single-nucleotide variants.
Single-marker and aggregate analyses of EUR and AFR samples. Logistic regression was performed on 57 SNVs. Gene-based analysis was performed on 35 exonic SNVs.

showed a nonsignificant excess in cases (EUR cases/controls: 0.138/0.119; AFR cases/controls: 0.243/0.167) mostly owing to novel variants in AFR subjects (cases/controls: 0.173/0.099). Three unrelated cases, but zero controls, were each found to have a single novel nonsense mutation: two for *DISC1* (truncating only the 'Es' splice variants) and one for *SLC6A4* (Supplementary Tables S2–S4). The ratio of nonsynonymous to synonymous variants was similar in cases and controls (1.25 vs 1.16).

In stage 2, we prioritized 254 of the 782 variants for technical replication, as they met at least one of the following criteria: (1) novel nonsense, missense or splice site variant, (2) novel intronic variant in $\geqslant 1$ EUR case, (3) novel variant with an odds ratio >2 in the EUR cohort and (4) dbSNP nonsense, missense or splice site variant in $\geqslant 1$ EUR case. Validation sequencing by Roche 454 revealed 225 true variants and 29 false positives (accuracy rate of 89%; Supplementary Figure S2).

In stage 3, we selected 92 out of the 225 SNVs (Supplementary Table S5) from stage 2 for genotyping in an independent sample of 2191 cases and 2659 controls (EUR and AFR). We included: (1) all novel nonsense, missense or splice site variants seen in $\geqslant 1$ case, (2) all variants seen in >1 case and (3) three nonsense variants observed in one case each. After genotyping 92 SNVs in the replication samples, 29 were monomorphic (22 of these were seen in only one case in stage 1), 6 had low-quality genotypes and 57 SNVs were tested for association with schizophrenia (logistic regression, separately for EUR and AFR subjects). Table 1 lists the SNVs with the smallest *P* value in each gene (complete results in Supplementary Table S6). No gene contained a SNV reaching the criteria for genome-wide significance ($P<5\times10^{-8}$). We then tested the aggregate effects of uncommon variants within a gene for 35 non-intronic SNVs with minor allele frequency (MAF) <0.01 (Table 1). No gene was significant following correction for multiple testing. For example, of the 20 uncommon variants in *DISC1* (Supplementary Figure S3), there were 32 minor alleles in EUR cases and 40 in EUR controls.

Thus, multistage resequencing of 10 schizophrenia candidate genes did not yield support for uncommon exonic variation. This result is consistent with common variation results that do not, to date, provide support for these genes despite a sample size of 21 856 individuals. The replication sample had 80% power to detect a genotypic relative risk of 3.2 in the EUR cohort and 5.1 in the AFR cohort, with an MAF of 0.01 and significance level of $P=5\times10^{-8}$. For a relaxed threshold ($P=0.001$), there would have been $>99\%$ power to detect a genotypic relative risk of 2.9 in the EUR cohort and 4.6 in the AFR cohort.

The stage 1 and 2 results hinted that *DISC1* might contain an excess of uncommon variants, and *DISC1* was thus the main focus in stage 3 replication. However, there was no evidence in our results to support the hypothesis that *DISC1* contains uncommon variants that are of relevance to schizophrenia, as no single-SNV or aggregate test approach was even of nominal significance. Although *DISC1* nonsense variants were present in 2 stage 1 cases and 0 controls, no additional cases or controls in the larger stage 3 sample had those particular nonsense mutations.

DISC1 has been the focus of dozens of genetic studies.[4] However, a recent comprehensive meta-analysis of common variation did not support its role in schizophrenia susceptibility.[5] To our knowledge, four groups have sequenced *DISC1* in cases with schizophrenia,[6–9] and all had discovery samples far smaller than reported here (34, 90, 198 and 288 cases). Of the association results in these studies, none met the criteria for genome-wide significance. Only one study had a replication component, and the initial finding did not replicate.[9] Therefore, despite employing a replication sample three times the size of the discovery sample, *DISC1* was not found to contain common or uncommon variants individually, or in aggregate associated with schizophrenia.

The results of this study suggest that classical schizophrenia candidate genes do not harbor uncommon coding region variations of etiological importance.

CONFLICT OF INTEREST

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